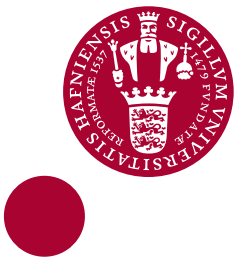

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Staphylococcus pseudintermedius colonization patterns and strain diversity in healthy dogs: A cross-sectional and longitudinal study

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ABSTRACT

This is the first large-scale study of *Staphylococcus pseudintermedius* colonization and diversity in healthy dogs where samples were collected over a long time and strains were identified by PCR according to the current taxonomy of the *S. intermedius* group and typed by a highly discriminatory method such as pulsed field gel electrophoresis (PFGE). A cross-sectional study of nasal, oral, perineal and inguinal carriage in 119 healthy dogs was followed by a longitudinal study where oral and perineal carriage was examined in 16 dogs for 10 times over a period of 1 year. Altogether we collected 762 samples and 285 *S. pseudintermedius* isolates, 182 of which were typed by PFGE to determine spatial and temporal strain diversity within individual carriers. In the cross-sectional study, *S. pseudintermedius* was isolated from at least one body site in 82 (69%) of the 119 dogs. The most frequent carriage sites were the perineum (66%) and the mouth (65%) followed by the nose (27%) and the groin (23%). PFGE analysis showed high heterogeneity among the isolates originating from different body sites of the same dog. Fifteen of the 16 dogs sampled longitudinally carried *S. pseudintermedius* in at least one sampling time, including six dogs that were negative in the cross-sectional study. Our results indicate that *S. pseudintermedius* carriage in dogs is more frequent and heterogeneous than *S. aureus* carriage in humans. This observation might reflect the hygienic standards and social behavior of the canine host, which facilitates transmission of this bacterium in the dog population.

1. Introduction

Staphylococcus pseudintermedius is an opportunistic pathogen residing on the skin and mucose of dogs. Infection usually arises when skin and mucosal barriers are altered by predisposing factors such as atopic dermatitis, medical and surgical procedures and/or immunosuppressive disorders (Bannoehr and Guardabassi, 2012). Colonization is a likely a risk factor for *S. pseudintermedius* infection since most dogs are infected with strains residing on their body (Sasaki et al., 2005; Pinchbeck et al., 2006; Fazakerley et al., 2010). The close relationship between colonization and infection is supported by the higher frequencies of carriage observed in dogs with atopic dermatitis compared to healthy dogs (Fazakerley et al., 2010).

Four cross-sectional studies conducted on healthy dogs have reported *S. pseudintermedius* carriage between 46% and 92% (Devriese and De Pelsmaecker, 1987; Griffeth et al., 2008; Hanselman et al., 2009; Rubin and Chirino-Trejo, 2011). A major limitation of cross-sectional studies is that they do not provide any information on the stability

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of carriage, i.e. positive dogs can be either persistent or intermittent carriers and negative dogs may be intermittent carriers. Thus, longitudinal studies are needed to classify dogs as persistent carriers, intermittent carriers and non-carriers. To date, only four longitudinal studies have been published on colonization patterns of *S. pseudintermedius* in healthy dogs (Cox et al., 1988; Saijonmaa-Koulumies and Lloyd, 2002; Saijonmaa-Koulumies et al., 2003; Hartmann et al., 2005). In all studies, more than half of the dogs tested were found to be persistent carriers. However, three studies were conducted on particular dog populations (i.e. pet therapy dogs or pregnant bitches and their litters) and in all studies *S. pseudintermedius* was not identified according to the current methods for species identification and strain diversity was either not assessed or assessed by poorly discriminatory methods.

The objective of this study was to explore colonization patterns and strain diversity of *S. pseudintermedius* in healthy dogs. A cross-sectional study in which screening of *S. pseudintermedius* was done by selective enrichment in 119 healthy dogs in the area of Copenhagen, Denmark, was followed by a longitudinal study of 16 dogs for a period of one year. All isolates were identified by a species-specific PCR and pulsed field gel electrophoresis (PFGE) was used as a high discriminatory method to assess strain diversity between body sites and between sampling times within individual dogs.

2. Materials and methods

2.1. Sampling

The cross-sectional study was performed between September and November 2010. A total of 102 healthy dogs were recruited among dogs attending the small animal hospital at the University of Copenhagen mainly for vaccination or deworming. In addition, 17 dogs belonging to homeless people in the Copenhagen area were sampled outdoor. All dogs were defined as healthy based on clinical examination and veterinary records (i.e. no documented infection and antibiotic treatment in last 6 months prior to sampling). Twenty-seven dogs belonged to multi-dog homes and six of them lived in the same household with another dog sampled. Samples were taken from the perineum (the adjacent skin area lateral to the anus), groin (right or left groin), mouth (superior gingival mucosa in the rostral part of the mouth), and the nose (nasal vestibulum) by veterinarians. Sterile cotton swabs (Dryswab™, MW&E, UK) were rubbed over the area for three seconds and analyzed within a few hours after collection.

The longitudinal study was started approximately 6 months after the cross-sectional study. Twenty dogs, including 10 carriers at multiple sampling sites and 10 non-carriers were selected for the longitudinal study but only 16 dog owners – seven negative and nine positive dogs – agreed to participate. Consent forms were sent to all dog owners together with a sampling kit and a detailed description of the sampling scheme and protocol. Based on the most prevalent positive body sites observed in the cross-sectional study, the owners were asked to collect samples from the perineum and mouth. Swabs were collected nine times over a period of 6 months, from March to September 2011. The sampling scheme included four weekly samples during the first month (days 1, 8, 15 and 22) and one monthly sample for the remaining 5 months (days 30, 60, 90, 120 and 150). The swabs were shipped in tubes containing transport medium and analyzed within 2–3 days after collection. According to the national regulations, the study was approved by the ethical committee of the University hospital, and informed consent was obtained from all dog owners prior to sampling.

2.2. Bacterial isolation and identification

Swabs were individually enriched overnight in 5 ml Mueller Hinton broth supplemented with 6.5% NaCl at 37 °C to select for staphylococci. Thereafter, 10 μl of enrichment culture was streaked onto 5% calf blood agar and incubated overnight. One colony displaying the characteristic appearance (non-pigmented, raised, medium sized and hemolytic) of *S. pseudintermedius* on blood agar was selected from each plate/sample, and confirmed to the species level by *nuc* PCR (Sasaki et al., 2010).

2.3. Definitions of carriers

Dogs positive for *S. pseudintermedius* at all sampling times ($n = 9$) were classified as persistent carriers. Intermittent carriers were distinguished between transient carriers that tested positive in at least three consecutive samples and sporadic carriers that were positive at only one or two of the nine sampling times. Non-carriers were defined as dogs testing negative at all sampling times.

2.4. PFGE

All *S. pseudintermedius* isolates from 41 dogs positive at multiple body sites in the cross-sectional study and all isolates from days 1, 30, 60, 120 and 150 of the longitudinal study were typed by PFGE according to the Harmony protocol (Murchan et al., 2003) with minor modifications. Briefly, overnight cultures in BHI were adjusted to OD 0.5 and incorporated into 1.0% (w/v) agarose plugs (Seakam Gold, Lonza, Rockland, ME, USA). After 18 h incubation with lysostaphin (1 mg/ml, Sigma–Aldrich, Germany), lysozyme (100 mg/ml, Sigma–Aldrich) and RNase (10 mg/ml, Sigma–Aldrich) at 37 °C, plugs were digested with proteinase K (20 mg/ml, Sigma–Aldrich) overnight at 50 °C. A 1.5 mm thick agarose section was digested with SmaI (10 U/μl, Fermentas GmbH, Germany) for 16 h at 25 °C. For optimization, 200 μM of thiourea (Sigma–Aldrich) was added to the running buffer and the gel (Alonso et al., 2005). The restriction fragments were separated by clamped homogenous electric field (CHEF) electrophoresis with a CHEF-DR III System (Bio-Rad Laboratories, USA) in a 1.1% (w/v) agarose gel using pulse time at 2 s followed by 5 s at 5.6 V/cm for 24 h. PFGE profiles were interpreted according to Tenover et al. (1995) to assess the genetic relationship of isolates originating from the same dog at different sampling sites and times.

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Table 1
Cross-tabulation of results from the four body sites from dogs positive with *S. pseudintermedius* at ≥1 sites ($n = 82$ dogs).

	Mouth		Nose		Groin	
	+	–	+	–	+	–
Perineum						
+	33	21	13	41	14	40
–	20	8	9	19	5	23
Mouth						
+			13	40	15	38
–			9	20	4	25
Nose						
+					6	16
–					13	47

For the cross-sectional study, gel images were analyzed by Gel Compar II (Applied Maths, Belgium) and cluster analysis was performed by UPGMA using dice similarity coefficient with optimization set at 0.5% and position tolerance at 1.5%. The strains were considered as identical or closely related when the PFGE band patterns were more than 93% similar.

2.5. Statistical analysis

The carrier prevalence was determined as the number of dogs positive for *S. pseudintermedius* in at least one of the four sample sites. To determine the optimal sampling strategy for the longitudinal study, a cross-tabulation of the test results from the four sites sampled in the cross-sectional study was done followed by pairwise comparison of the sites using the McNemar's test for paired proportions. Lastly, logistic regression was used to assess whether carriage was associated with previous history of skin problems, occurrence of multiple dogs in the household and homeless status.

3. Results

3.1. Cross-sectional study

A total of 82 of the 119 dogs (69%) carried *S. pseudintermedius* in at least one body site. Carriage at one, two, three and four body sites was observed in 48% (39/82), 30% (25/82), 16% (13/82) and 6% (5/82) of the positive dogs, respectively. The cross-tabulation of test-results from the four body sites is displayed in Table 1. Samples from perineum and mouth were most frequently positive ($n = 54$ and $n = 53$, respectively), followed by nose ($n = 22$) and groin ($n = 19$). A statistically significant association ($p < 0.001$) between the following body sites was observed: perineum–nose, perineum–groin, mouth–groin and mouth–nose. No significant association was observed between perineum and mouth ($p = 0.9$) and between nose and groin ($p = 0.6$). Combined sampling of the perineum and mouth resulted in detection of 90% (74/82) of carriers. Detection increased to 91% and 99% by additional sampling of the groin and the nose, respectively.

Twenty-five of the 119 dogs had previous history of skin and/or ear infections, and 21 of them (84%) were positive for *S. pseudintermedius*. Of the six multi-dog households, only one dog from each household was found to carry *S. pseudintermedius*. Similar carriage rates were found in dogs sampled at the University hospital (70%) and homeless dogs (65%). Logistic regression analysis showed that carriage was not associated with multi-dog homes ($p = 0.80$) and homeless status ($p = 0.67$), whereas a closer association was found between carriage and history of skin problems, despite not being statistically significant [$p = 0.08$, odds ratio = 2.8 (95% CI: 0.9; 9.1)].

3.2. Longitudinal study

Fifteen of the 16 dogs enrolled in the longitudinal study were positive in at least one sampling time, including six dogs that were negative in cross-sectional study (Table 2). *S. pseudintermedius* was isolated in all sampling times from six dogs (D7, D10, D11, D12, D13 and D16), which were classified as persistent carriers. Among the remaining 10 dogs, five (D3, D4, D9, D14 and D15) carried *S. pseudintermedius* in at least three consecutive sampling times (transient carriers), four (D2, D5, D6 and D8) were positive in only one or two sampling times (sporadic carriers) and one (D1) was negative in all sampling times (non-carrier).

3.3. Strain diversity

From the cross-sectional study, a total of 104 *S. pseudintermedius* isolates from 41 dogs colonized at two or more body sites were typed by PFGE. As illustrated by cluster analysis (Fig. 1), using a 93% similarity cut off value, 27 dogs (66%) carried identical or closely related strains at two or more body sites. Isolates from different dogs always displayed unrelated PFGE patterns.

In the longitudinal study, four of the six persistent carriers (D7, D10, D11 and D12) were found to carry two to three strains displaying genetically unrelated PFGE patterns (Fig. 2). Even higher genetic diversity was observed in one persistent carrier (D16), which carried seven unrelated strains according to the Tenover criteria (Fig. 2D). Similarly, four of the five transient carriers (D4, D9, D14 and D15) were found to carry at least two genetically unrelated strains. Among the four sporadic carriers, one (D2) carried the same strain in both samplings and the other two (D6 and D8) carried unrelated strains. Four isolates from the mouth of D14 were nontypable, whereas most isolates from the perineum of the same dog displayed the same PFGE pattern. Specific strain associations with body sites were observed in D2, D3, D4, D12 and D13.

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Table 2
Isolation of *S. pseudintermedius* from dogs during a 1-year period.

Dog ID	Sampling sites[a]	Sampling days										Carrier status[b]	PFGE types
		Cross sectional study	Longitudinal study[c]										
		Day −180	Day 1	Day 8	Day 12	Day 22	Day 30	Day 60	Day 90	Day 120	Day 150		
D1	M	–	–	–	–	–	–	–	–	–	–	N	NA
	P	–	–	–	–	–	–	–	–	–	–		
	G	–											
	N	–											
D2	M	–	–	–	–	–	–	–	–	+(1)	+(1)	S	1
	P	–	–	–	–	–	–	–	–	–	–		
	G	–											
	N	–											
D3	M	–	–	–	–	–	–	–	–	+(2)	–	T	2
	P	–	–	–	–	–	–	+(2)	+	+(2)	+(2)		
	G	–											
	N	–											
D4	M	–	+(3)	+	–	–	–	–	MS	–	–	T	3,4
	P	–	+(4)	+	+	+	–	+(4)	MS	–	+(4)		
	G	–											
	N	–											
D5	M	–	–	–	–	–	–	–	–	–	–	S	NA
	P	–	+	–	–	–	–	–	–	–	–		
	G	–											
	N	–											
D6	M	–	–	–	–	–	–	–	–	–	+(6)	S	5,6
	P	–	–	–	–	–	–	–	+(5)	–	–		
	G	–											
	N	–											
D7	M	–	+(7)	+	+	–	+(7)	–	+	+(8)	–	P	7,8
	P	–	–	+	+	+	–	+(8)	+	+(7)	+(8)		
	G	–											
	N	–											
D8	M	–	–	–	–	–	–	–	–	–	–	S	9, 10
	P	–	–	–	–	–	–	–	–	–	+(10)		
	G	–											
	N	+(9)											
D9	M	+(11)	+(12)	+	+	+	–	–	+	+(12)	+(12)	T	11, 12
	P	–	+(12)	+	–	–	–	–	–	–	–		
	G	–											
	N	–											
D10	M	+(13)	+(13)	+	+	+	+(13)	+(13)	+	+(14)	+(14)	P	13, 14
	P	–	+(14)	+	–	+	–	+(13)	+	+(14)	+(14)		
	G	–											

Table 2 (*Continued*)

Dog ID	Sampling sites[a]	Sampling days										Carrier status[b]	PFGE types
		Cross sectional study	Longitudinal study[c]										
		Day −180	Day 1	Day 8	Day 12	Day 22	Day 30	Day 60	Day 90	Day 120	Day 150		
	N	–											
D11	M	+(15)	+(15)	+	+	–	+(16)	+(15)	+	+(16)	+(17)	P	15, 16, 17
	P	–	–	+	+	+	+(16)	+(17)	+	+(17)	+(16)		
	G	–											
	N	–											
D12	M	+(19)	+(19)	+	–	+	+(19)	+(19)	+	–	+(18)	P	18, 19
	P	+(18)	+(18)	+	+	+	+(18)	+(18)	+	+(18)	+(18)		
	G	–											
	N	–											
D13	M	+(21)	+(21)	+	–	+	+(21)	+(21)	+	+(20)	–	P	20, 21
	P	+(20)	+(20)	+	+	+	–	+(20)	+	+(20)	+(20)		
	G	–											
	N	–											
D14	M	+(22)	–	+	+	–	+	+	+	–	+	T	22, 23
	P	+(22)	+(23)	–	–	–	–	+(23)	–	–	+(23)		
	G	–											
	N	+											
D15	M	+(25)	–	+	–	–	+(24)	+(27)	+	+(28)	+(28)	T	24, 25, 26, 27, 28
	P	+(24)	–	–	–	–	+(24)	–	+	–	+(28)		
	G	+(26)											
	N	–											
D16	M	+(30)	+(32)	+	+	+	+(31)	+(29)	+	+(34)	+(35)	P	29, 30, 31, 32, 33, 34, 35
	P	+(29)	–	+	+	+	+(29)	+(33)	+	–	–		
	G	+(30)											
	N	+(31)											

+, positive culture; –, negative culture; MS, missed sample; numbers within bracket indicate PFGE profile type.
[a] P, perineum; M, mouth; G, groin; N, nose.
[b] N, non-carrier; P, persistent carrier; S, sporadic carrier; T, transient carrier.
[c] Only mouth and perineal samples are taken in the longitudinal study.

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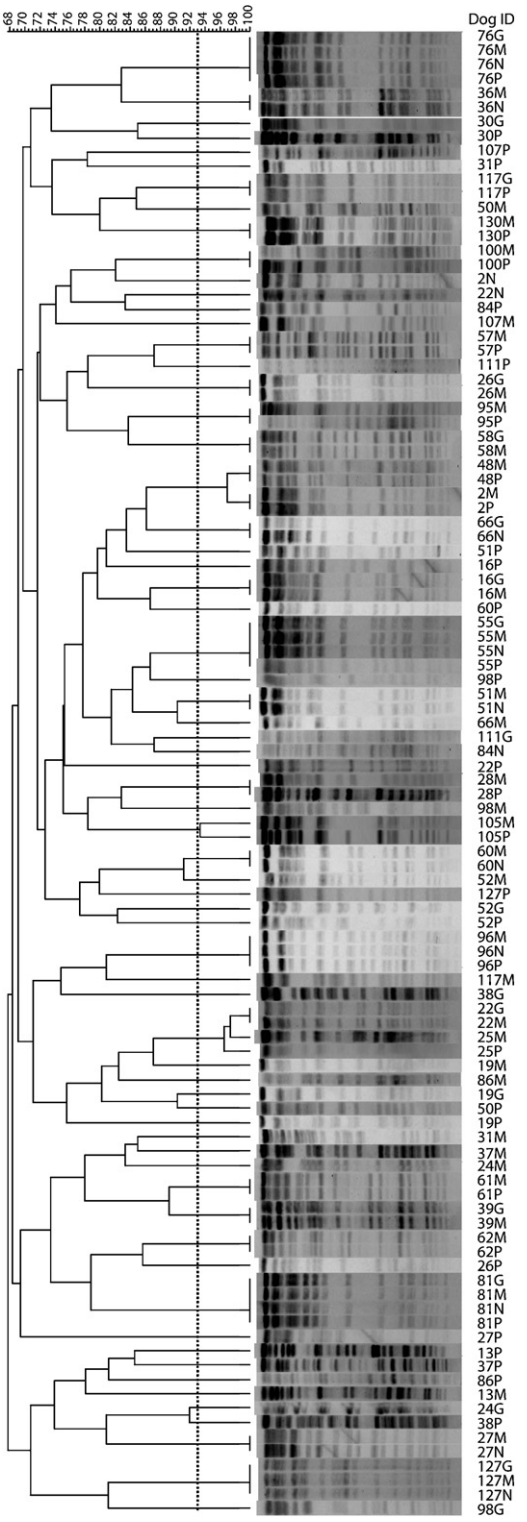

Fig. 1. Cluster analysis illustrating the genetic diversity of *S. pseudintermedius* isolated from ≥2 or more body sites from 41 dogs in the cross-sectional study. The dot line indicates the 93% similarity cut-off, above which PFGE profiles were considered as closely related. Each number indicates the individual dogs. P, perineum; M, mouth; G, groin; N, nose.

4. Discussion

A large-scale cross-sectional and longitudinal study was used to infer *S. pseudintermedius* colonization patterns and strain diversity in healthy dogs. The results confirmed high carriage rates reported by previous cross-sectional studies but revealed unanticipated genetic diversity between isolates from different body sites and different sampling times within individual dogs. High diversity was also observed at the population level since most dogs were found to carry genetically unrelated strains. Moreover, differently from previous longitudinal studies, intermittent carriage was shown to occur in more than half of the dogs despite the use of multiple body sites and selective enrichment for isolation of *S. pseudintermedius*.

We observed a 69% prevalence of *S. pseudintermedius* in healthy dogs, which is within the range (46–92%) observed in previous studies (Devriese and De Pelsmaecker, 1987; Griffeth et al., 2008; Hanselman et al., 2009; Rubin and Chirino-Trejo, 2011). The large variation between the prevalence data reported by different studies can be due to geographical, breed-specific and methodological differences. The clinical history of the study population is another factor to be considered as indicated by the borderline statistical significance observed in this study between *S. pseudintermedius* carriage and previous skin infection problems. The occurrence of *S. pseudintermedius* in healthy dogs is relatively higher than that of *S. aureus* in humans, where 20–30% of the population is positive for *S. aureus* (Wertheim et al., 2005). This difference might be related to low hygienic standards and the social behavior of dogs, which includes self-grooming and close contact with other dogs and the environment.

Oral and nasal cavity, perineum and groin are the body sites most frequently used for isolation of *S. pseudintermedius*. Other less commonly used body sites include the axilla, forehead, prepuce, vagina, external auditory canal and interdigital space (Devriese and De Pelsmaecker, 1987; Cox et al., 1988; Allaker et al., 1992; Harvey and Noble, 1998; Griffeth et al., 2008; Rubin and Chirino-Trejo, 2011). Obtaining multiple samples from an individual is time and resource demanding if large numbers of animals have to be tested. Our results indicate that perineum and mouth are the most frequently colonized body sites. The combination of the two samples allowed detection of 90% (75/82) of dog carriers, whereas oral and perineal samples alone could only detect 65% of carriers. Similarly to our study, Rubin and Chirino-Trejo (2011) demonstrated that nasal swabs are not suitable for detection of *S. pseudintermedius* in dogs and the same finding was reported by Windahl et al. (2012) for screening of methicillin-resistant *S. pseudintermedius* (MRSP). Simultaneous sampling of pharynx, perineum, the corner of the mouth and possible wounds was recommended for MRSP screening (Windahl et al., 2012).

The inherent limitation of cross-sectional studies is that individuals defined as carriers can be either persistent or intermittent, and non-carriers may be intermittent carriers (Wertheim et al., 2005). In the longitudinal study, we divided intermittent carriers into transient and sporadic carriers. Most dogs were either persistent, transient or sporadic carriers of *S. pseudintermedius*, and only one of the

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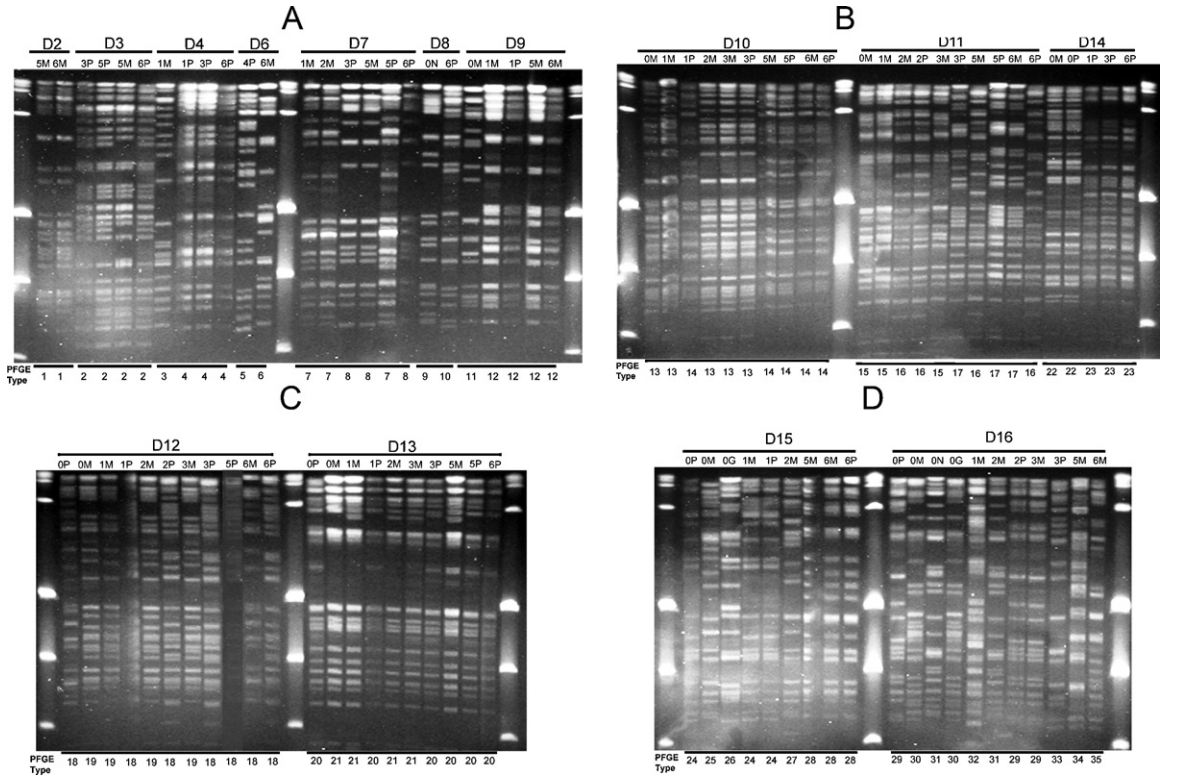

Fig. 2. PFGE gels of *S. pseudintermedius* isolated from dogs in the longitudinal study. The dogs are indicated by the numbers at the top of the figure (A, dogs D2–D9; B, dogs D10, D11 and D14; C, dogs D12 and D13; D, dogs D15 and D16). M, mouth; P, perineum; G, groin; N, nose. Numbers 0–6 before M/P/G/N indicate the sample number from representative days. 0, Day −180; 1, Day 1; 2, Day 30; 3, Day 60; 5, Day 120; 6, Day 150. The PFGE types are reported at the bottom.

16 dogs was consistently negative during the study period. Interestingly, the majority of the dogs scored as negative dogs in the cross-sectional study resulted to be transient or sporadic carriers in the longitudinal study. For determining human *S. aureus* carriage status, it has been shown that two quantitative nasal swab cultures taken 1 week apart are sufficient to identify persistent carriers but seven weekly nasal swabs were needed to distinguish intermittent from non carriers (Nouwen et al., 2004).

Irrespective of their carrier status (persistent, transient or sporadic), at least two unrelated strains were isolated from both the mouth and the perineum of most (11/15) carriers. As we only picked one colony per plate, it is possible that more than one clone was present in positive samples. This could explain why in some dogs (e.g. D10) one strain disappeared for some time and was re-isolated again later. The strain diversity observed in our longitudinal study is higher compared to previous longitudinal studies (Saijonmaa-Koulumies et al., 2003; Hartmann et al., 2005). Saijonmaa-Koulumies et al. (2003) found one or two of dominant RAPD genotypes in each of the three bitches tested and their puppies. Hartmann et al. (2005) reported that the majority of persistent and intermittent carriers were colonized with a single AP-PCR type and minor strain variations were observed by this typing method. It should be noted that PFGE is generally regarded as a more discriminatory method compared to the methods used by the previous longitudinal studies (AP-PCR and RAPD). This may explain the greater strain diversity observed in the present study. The high genetic diversity of *S. pseudintermedius* isolated from persistent dog carriers observed in this study is also in contrast to the typical colonization patterns of *S. aureus* in humans, where persistently individuals are colonized by a single strain type, whereas intermittent carriers may carry different strains overtime (VandenBergh et al., 1999). The dynamicity of *S. pseudintermedius* colonization in dogs is someway confirmed by a recent study on MRSP carriage (Windahl et al., 2012), where 14 of the 15 sampled dogs sampled were found to become MRSP-negative at all body sites within 12 months after infection.

5. Conclusion

S. pseudintermedius carriage is very common and heterogeneous in dogs. The dynamics of *S. pseudintermedius* colonization in dogs appears to be different compared to that of *S. aureus* colonization in humans in relation to both carriage frequency and strain diversity over time and between body sites. Mouth and perineum are the two most frequently colonized body sites and should be sampled in combination to increase the sensitivity of *S. pseudintermedius* screening in dogs.

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communication with the dog owners and collection of the samples. We also thank to Fanny Mauritzson and Alexandra Malmgren for their contribution to the study of dogs owned by homeless people.

References

Allaker, R.P., Lloyd, D.H., Bailey, R.M., 1992. Population sizes and frequency of staphylococci at mucocutaneous sites on healthy dogs. Vet. Rec. 130, 303–304.

Alonso, R., Martin, A., Pelaez, T., Marin, M., Rodriguez-Creixems, M., Bouza, E., 2005. An improved protocol for pulsed-field gel electrophoresis typing of *Clostridium difficile*. J. Med. Microbiol. 54, 155–157.

Bannoehr, J., Guardabassi, L., 2012. *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Vet. Dermatol., http://dx.doi.org/10.1111/j.1365-3164.2012.01046.x (Epub ahead of print).

Cox, H.U., Hoskins, J.D., Newman, S.S., Foil, C.S., Turnwald, G.H., Roy, A.F., 1988. Temporal study of staphylococcal species on healthy dogs. Am. J. Vet. Res. 49, 747–751.

Devriese, L.A., De Pelsmaecker, K., 1987. The anal region as a main carrier site of *Staphylococcus intermedius* and *Streptococcus canis* in dogs. Vet. Rec. 121, 302–303.

Fazakerley, J., Williams, N., Carter, S., McEwan, N., Nuttall, T., 2010. Heterogeneity of *Staphylococcus pseudintermedius* isolates from atopic and healthy dogs. Vet. Dermatol. 21, 578–585.

Griffeth, G.C., Morris, D.O., Abraham, J.L., Shofer, F.S., Rankin, S.C., 2008. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet. Dermatol. 19, 142–149.

Hanselman, B.A., Kruth, S.A., Rousseau, J., Weese, J.S., 2009. Coagulase positive staphylococcal colonization of humans and their household pets. Can. Vet. J. 50, 954–958.

Hartmann, F.A., White, D.G., West, S.E., Walker, R.D., Deboer, D.J., 2005. Molecular characterization of *Staphylococcus intermedius* carriage by healthy dogs and comparison of antimicrobial susceptibility patterns to isolates from dogs with pyoderma. Vet. Microbiol. 108, 119–131.

Harvey, R.G., Noble, W.C., 1998. Aspects of nasal, oropharyngeal and anal carriage of *Staphylococcus intermedius* in normal dogs and dogs with pyoderma. Vet. Dermatol. 9, 99–104.

Murchan, S., Kaufmann, M.E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C.E., Fussing, V., Salmenlinna, S., Vuopio-Varkila, J., El Solh, N., Cuny, C., Witte, W., Tassios, P.T., Legakis, N., van Leeuwen, W., van Belkum, A., Vindel, A., Laconcha, I., Garaizar, J., Haeggman, S., Olsson-Liljequist, B., Ransjo, U., Coombes, G., Cookson, B., 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. 41, 1574–1585.

Nouwen, J.L., Ott, A., Kluytmans-Vandenbergh, M.F., Boelens, H.A., Hofman, A., van Belkum, A., Verbrugh, H.A., 2004. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule". Clin. Infect. Dis. 39, 806–811.

Pinchbeck, L.R., Cole, L.K., Hillier, A., Kowalski, J.J., Rajala-Schultz, P.J., Bannerman, T.L., York, S., 2006. Genotypic relatedness of staphylococcal strains isolated from pustules and carriage sites in dogs with superficial bacterial folliculitis. Am. J. Vet. Res. 67, 1337–1346.

Rubin, J.E., Chirino-Trejo, M., 2011. Prevalence, sites of colonization, and antimicrobial resistance among *Staphylococcus pseudintermedius* isolated from healthy dogs in Saskatoon, Canada. J. Vet. Diagn. Invest. 23, 351–354.

Saijonmaa-Koulumies, L.E., Lloyd, D.H., 2002. Colonization of neonatal puppies by *Staphylococcus intermedius*. Vet. Dermatol. 13, 123–130.

Saijonmaa-Koulumies, L.E., Myllys, V., Lloyd, D.H., 2003. Diversity and stability of the *Staphylococcus intermedius* flora in three bitches and their puppies. Epidemiol. Infect. 131, 931–937.

Sasaki, A., Shimizu, A., Kawano, J., Wakita, Y., Hayashi, T., Ootsuki, S., 2005. Characteristics of *Staphylococcus intermedius* isolates from diseased and healthy dogs. J. Vet. Med. Sci. 67, 103–106.

Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, S., Kawakami, T., Fukata, T., Hiramatsu, K., 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J. Clin. Microbiol. 48, 765–769.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial typing. J. Clin. Microbiol. 33, 2233–2239.

VandenBergh, M.F., Yzerman, E.P., van Belkum, A., Boelens, H.A., Sijmons, M., Verbrugh, H.A., 1999. Follow-up of *Staphylococcus aureus* nasal carriage after 8 years: redefining the persistent carrier state. J. Clin. Microbiol. 37, 3133–3140.

Wertheim, H.F., Melles, D.C., Vos, M.C., van Leeuwen, W., van Belkum, A., Verbrugh, H.A., Nouwen, J.L., 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect. Dis. 5, 751–762.

Windahl, U., Reimegård, E., Holst, B.S., Egenvall, A., Fernström, L., Fredriksson, M., Trowald-Wigh, G., Andersson, U.G., 2012. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs—a longitudinal study. BMC Vet. Res. 8, 34.